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(54) DIAGNOSTIC AGENT

(71) We, BOEHRINGER MANNHEIM GMBH, of Mannheim-Waldhof, Germany, a Body Corporate organised under the laws of Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with diagnostic agents for the detection of components in liquids, especially in body fluids, comprising an absorbent carrier impregnated with reagents.

Test strips impregnated with suitable reagents have been in use for a long time. pH indicator papers are used very widely but other reagent papers are also employed. In recent years, test papers have achieved great importance for the detection of glucose, protein, nitrite and the like in body fluids, for example in urine and blood, because they enable the physician to carry out a rapid and simple diagnosis of metabolic disturbances outside the laboratory.

For the various tests, a large number of absorbent carriers has been proposed, for example, wood, asbestos, gypsum, glass fibre felts, synthetic resin fleeces and the like but in actual practice filter paper is used almost exclusively for the commercially available test strips. The reason for this is that, in addition to the cheapness and ease of working up of paper, the reagents on the cellulose fibres of the paper have an especially good reactivity.

Although, as just pointed out, filter paper is usually the best carrier material, there are chemical test reactions which cannot be carried out on paper. Quite apart from test reagents which, in a state of fine division, are unstable in air and, therefore, cannot be applied to a carrier by impregnation, there is a number of test reagents which destroy paper fibres and make the paper brittle. Such reagents include, for example, strongly alkaline, strongly acidic and oxidising substances and especially reagent mixtures with a high salt content.

Tests which are especially important for medical diagnosis but which cannot be carried out or cannot be carried out satisfactorily with the use of test strips include, for example, Legal's test for ketonic bodies and Ehrlich's test of pyrrole bodies. Legal's test requires, for a satisfactory functioning, a high concentration of a strongly alkaline buffer in the reaction solution and Ehrlich's test requires a high concentration of a weakly acidic compound, for example, oxalic acid or potassium bisulphate.

Filter papers which have been impregnated with the high salt concentrations necessary for these tests admittedly show a rapid and sensitive reaction with the substrates in question but are so hard and brittle that they cannot be bent, folded or cut up without breaking and crumbling. Since not only in the production but also in the transport and use of such strips, mechanical stresses constantly occur, the usefulness of such strips is severely limited.

Attempts to make such papers mechanically more stable by laminating on to a synthetic resin film were unsuccessful because the hard paper, upon bending or folding, again separated or crumbled away from the film. Cellulose fleeces strengthened with synthetic resin fibres, for example, with polyvinyl chloride or polyester fibres, also have not proved to be advantageous because they still did not possess a sufficient degree of stability.

The complete replacement of cellulose by more stable synthetic resin fibres, for example by polypropylene fibres, such as has been proposed in German Patent Specification No. 2,007,013 for a bilirubin test, also do not prove to be successful in the case of the above-mentioned tests but only showed very

weak colour changes which, in the lower range of concentrations, could no longer be evaluated.

Microscopic investigations have shown that the important difference between the cellulose fibres of paper and synthetic resin fibres is that the cellulose fibres swell during the impregnation and a part of the reagents is embedded or incorporated into the fibres, whereas in the case of synthetic resin fibres, the reagents are only deposited on the surfaces of the fibres.

After laborious and fruitless experiments with a very large variety of absorbent materials, especially with various synthetic resin fleeces, we have now, surprisingly, found that felts or fleeces which consist entirely or preponderantly of synthetic polyamides differ significantly from all of the other materials tested. Only these fleeces or felts based on synthetic polyamides provide the final test strips with a sufficient degree of stability, without destroying the reactivity and sensitivity of the test reagents.

It is not known why synthetic polyamides differ in this manner from other synthetic resins, for example from polyesters and polyvinyl chlorides. The difference is extremely surprising because the reagents are also not embedded or incorporated into the polyamide fibres.

As synthetic polyamides to be used according to the present invention, there can be employed not only those of the nylon type, made from dicarboxylic acids and diamines, but also those of the perlon type, made from ω-aminocarboxylic acids. As mixture components in mixed felts and fleeces, it is especially preferred to use polyester fibres but fibres of other synthetic resins, for example of polyvinyl chloride and the like, can also be admixed in amounts of less than 50%.

The felts and fleeces can be produced not only by wet depositing but also by dry-depositing, the fibres can be oriented or lie at random and they can be connected thermally or by binding agents or they can be needled.

The felt or fleece selected depends essentially upon the nature of the reagents to be used for the impregnation thereof. From the large number of commercially-available felts and fleeces the best one can easily be determined by a few simple preliminary experiments.

Thus, in the case of impregnation with acidic salts, for example with potassium bisulphate in the case of the urobilinogen test, it is recommended to use a mixed felt or fleece containing polyester fibres. On the other hand, in the case of basic salts, such as are used for the ketonic body test, pure synthetic polyamide felt or fleece has proved to be the best. In special cases, a mixed felt or fleece of synthetic polyamide fibres and cellulose fibres can also be used.

The thickness and weight per unit area of the felt or fleece used can be varied. However, very thin or light felts and fleeces take up very little reagent and thus have a poorer reactivity. Thick or voluminous felts and fleeces, on the other hand, take up large amounts of reagents and it is thus more difficut to work them up. Hence again, the most suitable felt or fleece for any particular case can be easily determined by a few simple preliminary experiments.

The felt or fleece can be impregnated in conventional manner. However, in order to improve wettability, it is sometimes desirable either to add a wetting agent to the impregnation solution or first to impregnate the felt or fleece with a wetting agent.

The impregnated felt or fleece is then dried in the usual manner. If desired, it can be cut up into narrow strips and used directly or, as still smaller pieces, can either be stuck on to a handle of synthetic resin or, according to our British Patent Specification No. 960,803, can be sealed between synthetic resin films or, according to our copending Patent Aplication No. 9796/72 (Serial No. 1,349,623), can be sealed between a synthetic resin film and a synthetic resin mesh.

The following Examples are given for the purpose of illustrating the present invention, the properties of the fleeces and felts used in the following Examples being summarised in Table 4 given hereinafter:—

Example 1.

Urobilinogen test.

The materials set out in the following Table 1 are impregnated with an aqueous solution containing, per 100 ml., 20 g. potassium bisulphate and 0.2 g. *p*-dimethylaminobenzaldehyde. The properties of the impregnated test strips, as well as their reaction with urobilinogen-containing urine, are also set out in the following Table 1.

TABLE 1

material and material number	stability	reaction
filter paper (1)	very brittle becomes grey	good uniform
polyester fleece (9)	stable	weak non-uniform
polyester-polyamide fleece (8)	stable	very good uniform

Example 2.

Ketonic body test.

The materials set out in the following Table 2 are impregnated with Solution I, dried, impregnated with Solution II and again dried.

Solution I

trisodium phosphate dodecahydrate	21.0 g.
disodium hydrogen phosphate dihydrate	11.2 g.
glycocoll	18.7 g.
distilled water	ad 100.0 ml.

Solution II

sodium nitroferricyanide dihydrate	0.9 g.
polyvinylpyrrolidone-vinyl acetate copolymer (50% solution in ethanol)	6.5 ml.
organic phosphate ester of anionic surface-active agent (10% solution in ethanol)	1.7 ml.
dimethyl sulphoxide	38.0 ml.
ethanol	18.5 ml.
distilled water	ad 100.0 m.l

The properties of the impregnated test strips and the reactions with urine containing acetoacetate or of acetone are also set out in the following Table 2.

TABLE 2

material and material number	stability	reaction
filter paper (1)	very brittle	very good uniform
polyester fleece (9)	stable	weak non-uniform
polyamide fleece (5)	stable	good uniform

Example 3.

Ketone body test.

The materials set out in the following Table 3 are impregnated with Solution I, dried, again impregnated with Solution II and again dried.

Solution I

tetrasodium ethylenediaminetetraacetate	38.5 g.
glycocoll	18.7 g.
distilled water	ad 100.0 ml.

Solution II

sodium nitroferricyanide dihydrate	1.0 g.
dimethyl formamide	40.0 ml.
methanol	ad 100.0 ml.

The following Table 3 also shows the mechanical properties and the reactions of the test strips with urine which contains acetoacetate or of acetone.

TABLE 3

material and material number	properties	reaction
filter paper (1)	very brittle	very good
cellulose-cottonwool fleece (2)	very brittle	still good
regenerated cellulose-polyvinyl chloride fleece (3)	rather brittle	good
regenerated cellulose-polyamide fleece (4)	scarcely brittle	very good
polyamide fleece (5)	stable	very good
polyamide fleece (6)	stable	good
polyamide fleece (7)	stable	still good
polyester-polyamide fleece (8)	stable	good
polyester fleece (9)	stable	week
polyester fleece (10)	stable	weak

The following Table 4 gives the properties and description of the various carrier materials used in the above Examples:

TABLE 4

material No.	material	type	producer	synthetic resin monomers and average mol. wt.	working up	thickness in mm.	wt. per unit area in g/m²
1	filter paper	23SL	Schleicher & Schull	—	random, with wet tear strength agent	0.45	230
2	cellulose-cottonwool fleece (7:3)	Paratex III/50	Lohmann KG	—	longitudinally laid with binding agent	0.2	190
3	regenerated cellulose-polyvinyl chloride fleece (95:5)	VS 446	Binzer	vinyl chloride M.W. about 100,000	random, with binding agent	0.5	100
4	regenerated cellulose-polyamide fleece (1:1)	(sample)	Binzer	ε-caprolactam M.W. about 22,000	random, with binding agent	1.0	150
5	polyamide fleece	V 27835 (sample)	C. Fru-denberg	ε-caprolactam M.W. about 20,000	longitudinally and transversely laid, thermally strengthened	0.35	100
6	polyamide fleece	N 933c (sample)	C. Freu-denberg	ε-caprolactam M.W. about 20,000	longitudinally and transversely laid, thermally strengthened	0.5	95

TABLE 4 (Continued)

material No.	material	type	producer	synthetic resin monomers and average mol. wt.	working up	thickness in mm.	wt. per unit area in g/m²
7	polyamide fleece	FT 2114	C. Freudenberg	ε-caprolactam M.W. about 20,000	longitudinally and transversely laid, thermally strengthened	0.25	80
8	polyester-polyamide fleece (1:1)	Suprotex	Kalle	terephthalic acid-ethylene glycol M.W. about 18,000 adipic acid-hexamethylene-diamine, M.W. about 20,000	longitudinally and transversely laid, thermally strengthened needled, without binding agent	1.5	300
9	polyester fleece	E 5209 (sample)	Kalle	terephthalic acid-ethylene glycol M.W. about 18,000	needled, thermally strenghtened	0.35	250
10	polyester fleece	H 1015	C. Freudenberg	terephthalic acid-ethylene glycol M.W. about 18,000	longitudinally and transversely laid, thermally strengthened	0.25	170

In our copending Application No. 53519/72 (Serial No. 1,369,138), there is described and claimed a process for the production of a diagnostic agent for the detection of ketones, wherein an absorbent carrier, for example filter paper or cellulose fleece reinforced with synthetic polyamide fibres, is first impregnated with a solution consisting of an amino acid and tetrasodium ethylenediaminetetraacetate (as buffer) in water and dried and is then impregnated with a solution consisting of sodium nitroferricyanide in dimethyl formamide and optionally also a C_1—C_4 alcohol, and again dried. This co-pending Application also claims a diagnostic affect made by the claimed process. In the present Application, we make no claim to the diagnostic agent claimed in our co-pending Application No. 53519/72 (Serial No. 1,369,138).

Subject to the above disclaimer, WHAT WE CLAIM IS:—

1. A diagnostic agent for the detection of the components of liquids, comprising a felt or fleece made wholly or preponderantly of synthetic polyamide fibres and impregnated with reagents.

2. A diagnostic agent according to claim 1, wherein the felt or fleece contains polyester fibres, polyvinyl chloride fibres or cellulose fibres in an amount of less than 50%.

3. A diagnostic agent according to claim 1 or 2, wherein the felt or fleece used has been made by wet or dry depositing, the fibres of which are oriented or lie at random.

4. A diagnostic agent according to any of the preceding claims, wherein the fibres of the felt or fleece are connected thermally or by binding agents.

5. A diagnostic agent according to any of the preceding claims, wherein a needled felt or fleece is used.

6. A diagnostic agent according to any of the preceding claims, whenever impregnated with the reagents for Legal's test for ketonic bodies.

7. A diagnostic agent according to any of claims 1 to 5, whenever impregnated with the reagents for Ehrlich's test for urobilinogen.

8. A diagnostic agent according to any of the preceding claims, whenever in the form of a narrow strip or in the form of a small piece which is stuck on to a synthetic resin handle or sealed between two synthetic resin films or sealed between a synthetic resin film and a synthetic resin mesh.

9. A diagnostic agent according to claim 1, substantially as hereinbefore described and exemplified.

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